

no. 23888-7008. However, the Commissioner is not authorized to charge the cost of the issue fee to the Deposit Account.

Respectfully submitted,

Date: July 23, 2002

By: Antoinette F. Konski  
Antoinette F. Konski  
Reg. No. 34,202

Bingham McCutchen LLP (formerly McCutchen, Doyle, Brown & Enersen LLP)  
Three Embarcadero Center, Suite 1800  
San Francisco, CA 94111-4067  
Telephone: (650) 849-4950  
Facsimile: (650) 849-4800



COPIES PAID  
ORIGINALLY FILED

RECEIVED

AUG 06 2002

TECH CENTER 1600/2900

VERSION WITH MARKINGS TO SHOW CHANGES MADE

In the Specification:

Paragraph 0004 at page 2 has been amended as follows:

[0004] Angiogenesis normally occurs in a carefully controlled manner during embryonic development, during growth, and in special cases such as wound healing and the female reproductive cycle (Wilting and Christ, (1996) *Naturwissenschaften* 83:153-164; Goodger and Rogers, (1995) *Microcirculation* 2:329-343; Augustin et al., (1995) *Am. J. Pathol.* 147(2):339-351). Some of the important steps in the process of angiogenesis are: 1) growth factor (i.e. vascular endothelial growth factor, VEGF) signaling; 2) matrix metalloproteinases (MMP) and VEGF receptor interaction; 3) endothelial cell migration to site of growth factor signaling; and 4) endothelial cell tubule formation. Pathological angiogenesis play a central role in a number of human diseases including tumor growth and metastatic cancer, diabetic retinopathy, rheumatoid arthritis, and other inflammatory diseases such as psoriasis (Folkman, (1995) *Nature Med.* 1(1):27-31; Polverini, (1995) *Crit. Rev. Oral Biol. Med.* 6(3):230-247; Walsh, (1999) *Rheumatology* 38(2):103-112; Healy et al., (1998) *Hum. Reprod. Update* 4(5):736-[396] 740). In these cases, progression of disease is driven by persistent unregulated angiogenesis. For example, in rheumatoid arthritis, new capillary blood vessels invade the joints and destroy the cartilage. In diabetic retinopathy, capillaries in the retina invade the vitreous, bleed and cause blindness. [In diabetic retinopathy, capillaries in the retina invade the vitreous, bleed and cause blindness.] Significantly, tumor growth and metastasis are angiogenesis dependent. Most primary solid tumors go through a prolonged avascular state during which growth is limited to approximately 1-2 mm in diameter. Up to this size, tumor cells can obtain the necessary oxygen and nutrient supply by passive diffusion. These microscopic tumor masses can eventually switch on angiogenesis and recruit surrounding blood vessels to begin sprouting capillaries that vascularize the tumor mass, providing the potential for continuing expansion of the tumor and metastasis of malignant cells to distant locations. Although significant progress has been made in understanding the biological events that occur during pathological angiogenesis, there are presently no effective pharmaceutical compounds that are useful for controlling angiogenesis *in vivo*. Thus, effective therapies capable of controlling angiogenesis have the potential to alleviate a significant number of human diseases.

Paragraph 0072 at page 18 has been amended as follows:

### **Example 3**

#### *Endothelial Cell Assays ("CPAE")*

The assays were carried out according to the procedures of Connolly [Connolly], et al. (1986) Anal. Biochem. **152**:136-140[4] with modifications (Liang and Wong (1999) ANGIOGENESIS: FROM THE MOLECULAR TO INTEGRATIVE PHARMACOLOGY edited by Maradoudakis, Kluwer Academic/Plenum. Publishers, New York). Calf Pulmonary Arterial Endothelial (CPAE) cells are plated at 10,000 cells per well in 24 well culture plates. After growth incubation at 37°C, 5% CO<sub>2</sub> for about 60 hours, a dosage of the sample is added (about 50µl to about 100µl) to each sample well and re-incubated for 30 minutes. After incubation, cells are assayed visually under an inverted microscope to detect the presence of cells and through the use of the ECC assay. Both methods are used to detect the presence or absence of endothelial cells in each well. Control cells containing no sample were used and grew normally.

Paragraph 0074 at page 19 has been amended as follows:

### **Example 5**

#### *MMP Assay*

P.C. Brooks, et. al. (1996) in "Localization of matrix metalloproteinase MMP-2 to the surface of invasive cells by interaction with integrin  $\alpha v \beta 3$ ," (1996) Cell **85**:683-93 describes an *in vitro* assay on matrix metalloproteinase and  $\alpha v / \beta 3$  integrin interaction. The effects of the experimental sample on the MMP-2/ $\alpha v \beta 3$  integrin complex determines if the sample's mechanism of action involves any disruption of this segment of the angiogenic pathway. This involves testing if the experimental sample can inhibit the interaction of MMP-2 with the  $\alpha v \beta 3$  integrin. Initially, this is done via an ELISA using antibodies for MMP-2 and testing the binding of these antibodies to the sample. Further studies are pursued if a positive result occurs. TIMP-2 (Tissue Inhibitor of Matrix Metalloprotease-2), a known natural inhibitor of MMP-2, is used as the control.